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J.K.+C

JANENE PEISKER
TEAM LEADER EXAMINATION
SUPPORT AND SALES

NOVEL CARBOHYDRATE BASED ANTI-BACTERIALS

FIELD OF THE INVENTION

The Invention relates to disaccharides useful as antibacterial agents, methods of screening such compounds for anti-bacterial activity, methods of treatment using such compounds, and pharmaceutical compositions of such compounds

BACKGROUND OF THE INVENTION

Bacteria have a great ability to generate resistance to drugs through lateral gene transfer, mutation of enzymes, or by expressing enzymes which actively pump out the drug or break it down. Over the past 10 years resistance to existing drugs has become a significant problem in many countries. No new antibacterial drugs have been developed over the past 15 years. Vancomycin is currently the drug of last resort to combat the multidrug resistant Gram-positive bacteria. In many places vancomycin-resistant Staphylococcus Aureus and Enterococci (VRE) have been discovered. There is thus a desperate need for a new antibacterial drug to replace the drug of last resort.

There are a host of cytoplasmic targets for the development of new antibacterials, such as gyrase, protein synthesis inhibitors, muramyl cascade inhibitors and many more. The major hurdle in designing such drugs is that in addition to enzyme based activity these drugs need to cross the bacterial cell wall to exert their antibacterial effect. On the other hand, enzymes involved in the stage III synthesis of the bacterial cell wall exist on the cell wall exterior, and therefore drugs inhibiting these enzymes can exert their bactericidal or bacteriostatic effect without having to cross the cell wall. Penicillin, cephalosporin and vancomycin are drugs that act on the transpeptidase enzymes which control the final steps in the peptidoglycan biosynthesis. Moenomycin is known to act on the transglycosylase enzymes, which are similarly involved in the polymerization of disaccharide precursors. Moenomycin displays very high potency at MIC level, and is used in animal feed as a growth promoter.

Moenomycin is a lipid-linked pentasaccharide. Through extensive SAR experiments it was realised that smaller fragments of moenomycin were

capable of exerting antibacterial activity. Disaccharide fragments of moenomycin still display antibacterial activity, but are not sufficiently stable to be useful drugs. On the basis of this, Sofia and coworkers discovered a new series of disaccharides, carrying aromatic substituents in well defined positions around the disaccharide, which displayed significant MIC activity [WO0064915 and WO9926596].

A further class of disaccharide molecules, based on a sub-structure of vancomycin was shown to have antibacterial activity against vancomycin resistant bacteria. This class of molecules was subsequently demonstrated to contain transglycosylase inhibitors, and were not transpeptidase inhibitors as is vancomycin itself [WO9853813].

SUMMARY OF THE INVENTION

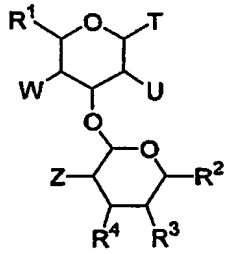
The present invention is directed to carbohydrate compounds and particularly disaccharide like moieties that display antibacterial activity against Gram-positive bacteria.

The present invention is also directed to method for the treatment of humans or animals using such compounds in a therapeutically effective amount.

The present invention is also directed to pharmaceutical preparations containing such compounds.

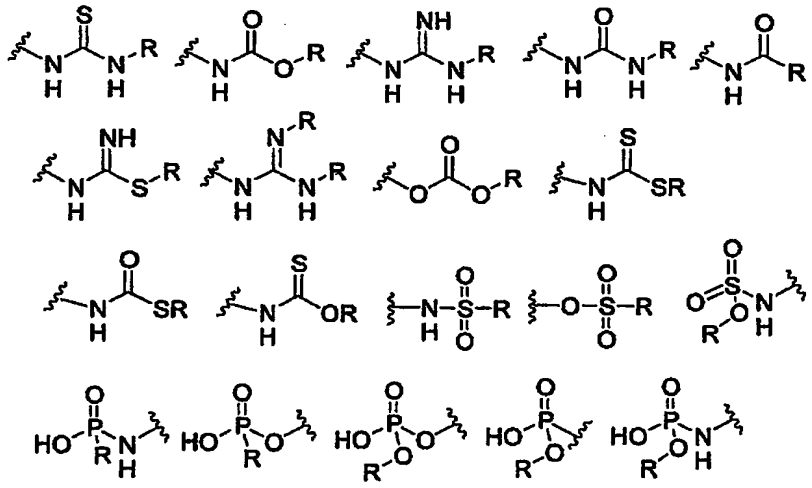
The present invention is also directed to a method of screening such compounds for anti-bacterial activity by contacting the compounds with a Gram-positive bacteria and monitoring the growth or growth inhibition of the bacteria.

In a first aspect, the invention provides disaccharide compounds of General Formula I,

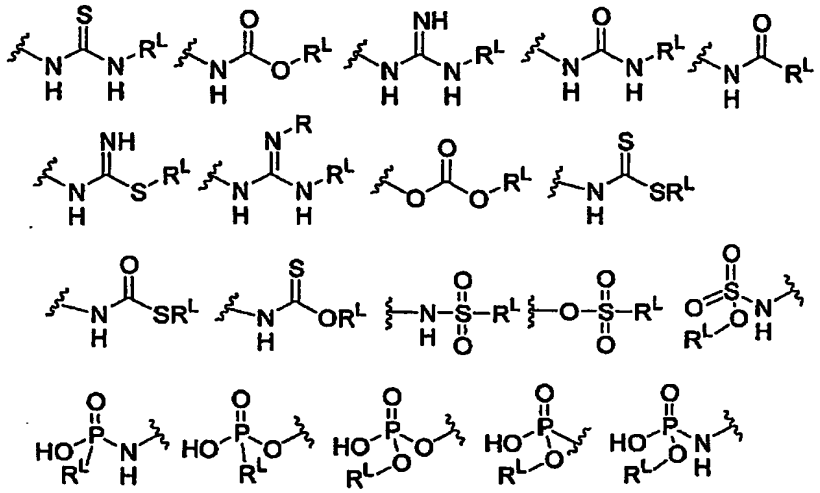


General Formula I

Wherein the pyranose rings may be of any configuration, T is either R or -XR, where X is defined as oxygen, sulphur, NHC(O)-, and wherein R is selected from the non-limiting set comprised of H, or an alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, arylalkyl or heteroarylalkyl of 1 to 20 atoms which is optionally substituted, and can be branched or linear. Typical substituents include but are not limited to OH, NO, NO_2, NH_2, N_3, halogen, CF_3, CHF_2, CH_2F, nitrile, alkoxy, aryloxy, amidine, guanidiniums, carboxylic acid, carboxylic acid ester, carboxylic acid amide, aryl, cycloalkyl, heteroalkyl, heteroaryl, aminoalkyl, aminodialkyl, aminotrialkyl, aminoacyl, carbonyl, substituted or unsubstituted imine, sulfate, sulfonamide, phosphate, phosphoramide, hydrazide, hydroxamate, hydroxamic acid, heteroaryloxy, aminoalkyl, aminoaryl, aminoheteroaryl, thioalkyl, thioaryl or thioheteroaryl, which may optionally be further substituted, U and Z independently selected from OR, NHR, NHR(R) (where R may be the same or different), or the following non-limiting set,

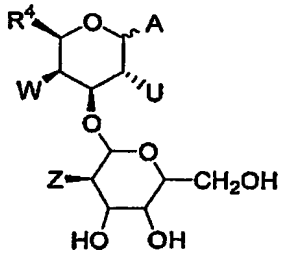


R^1 and R^2 are independently selected from H, CH_3, CH_2XR, and C(O)NHR, R^3 and R^4 are independently selected from H, OH, OR, NHCOR, and W is independently selected from OR^L, NHR^L, NR^LR, or the following the following non-limiting set,



Wherein R^L is a substituted or unsubstituted, linear or branched, saturated or unsaturated C3 to C55 alkyl, heteroalkyl, arylalkyl, alkylaryl chain. Substituents may include but are not limited to acidic groups such as carboxylic acids, sulfonic acids, phosphoric acids, tetrazoles, or other carboxylic acid mimetics.

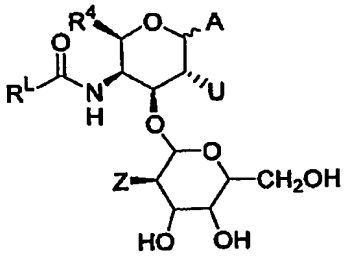
In one embodiment the invention provides disaccharide compounds of General Formula II,



General Formula II

Wherein the disaccharide linkage is alpha or beta, A is defined as hydrogen, OR or SR, and R, U, W, Z and R^4 are defined as in General Formula I.

In a more preferred embodiment, the invention provides disaccharide compounds of General Formula III,



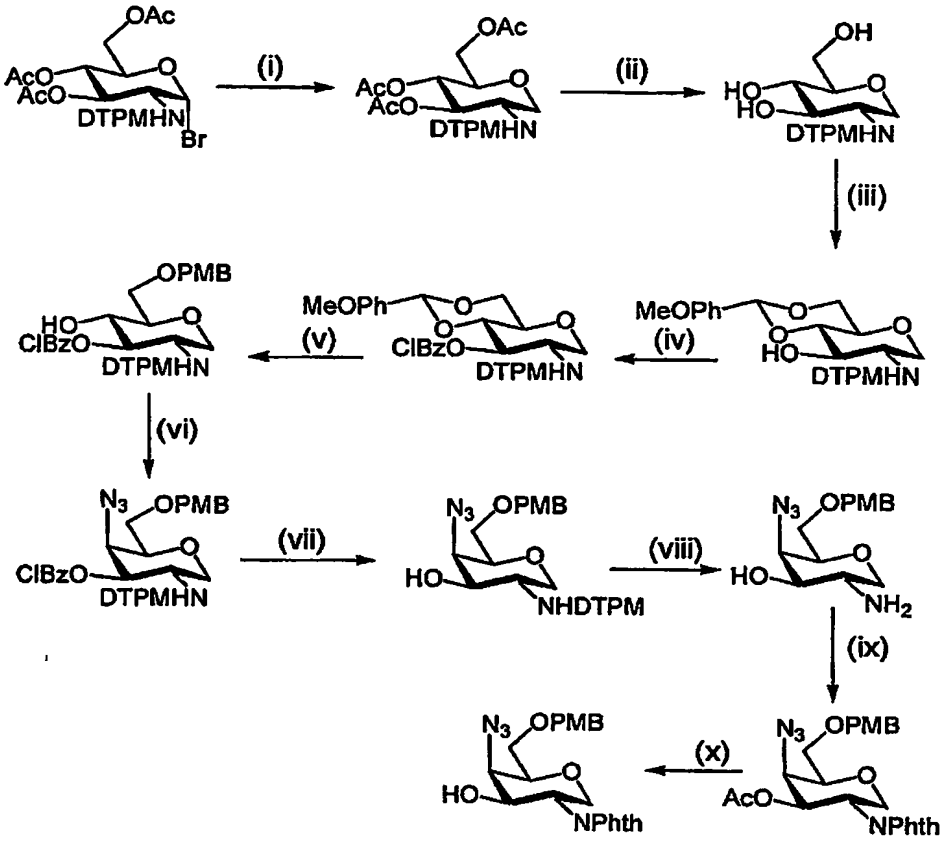
General Formula III

Wherein A is defined as in General Formula I, and U, Z, R^L and R^4 are defined as in General Formula I.

In a second aspect, the invention provides a method of treatment of a bacterial infection, comprising the step of administering an effective amount of a compound of the first aspect, to a subject in need of such treatment. The subject may be a human, or may be a domestic, companion or zoo animal.

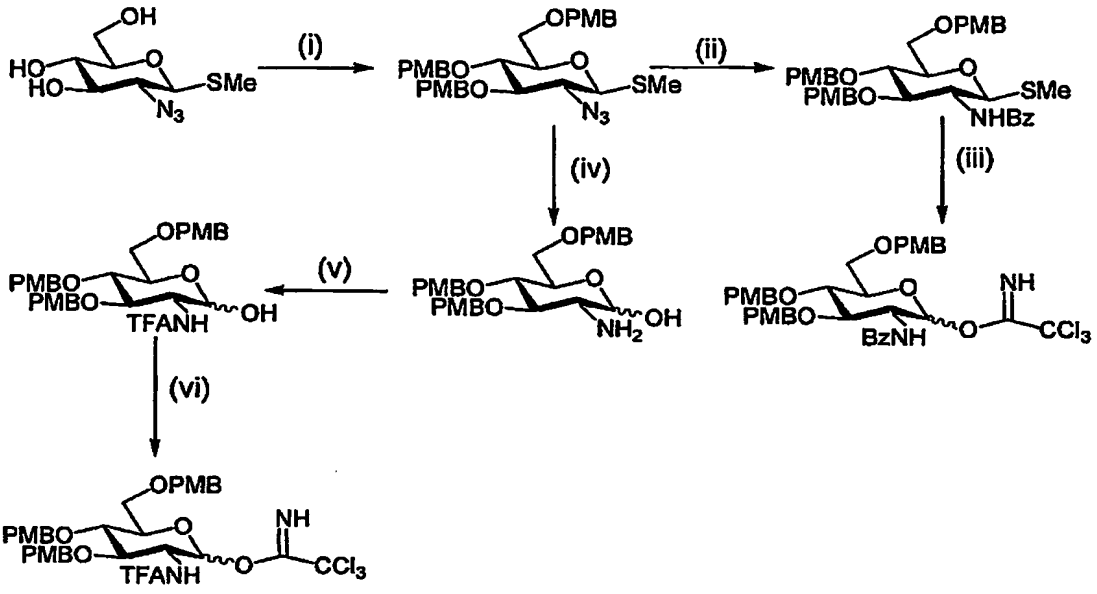
Methods and pharmaceutical carriers for preparation of pharmaceutical compositions are well known in the art, as set out in textbooks such as Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Company, Easton, Pennsylvania, USA.

Example 1: Synthesis of 1-deoxy-1-H-2,4-deoxy-2,4-*N*-Galactosyl Acceptor



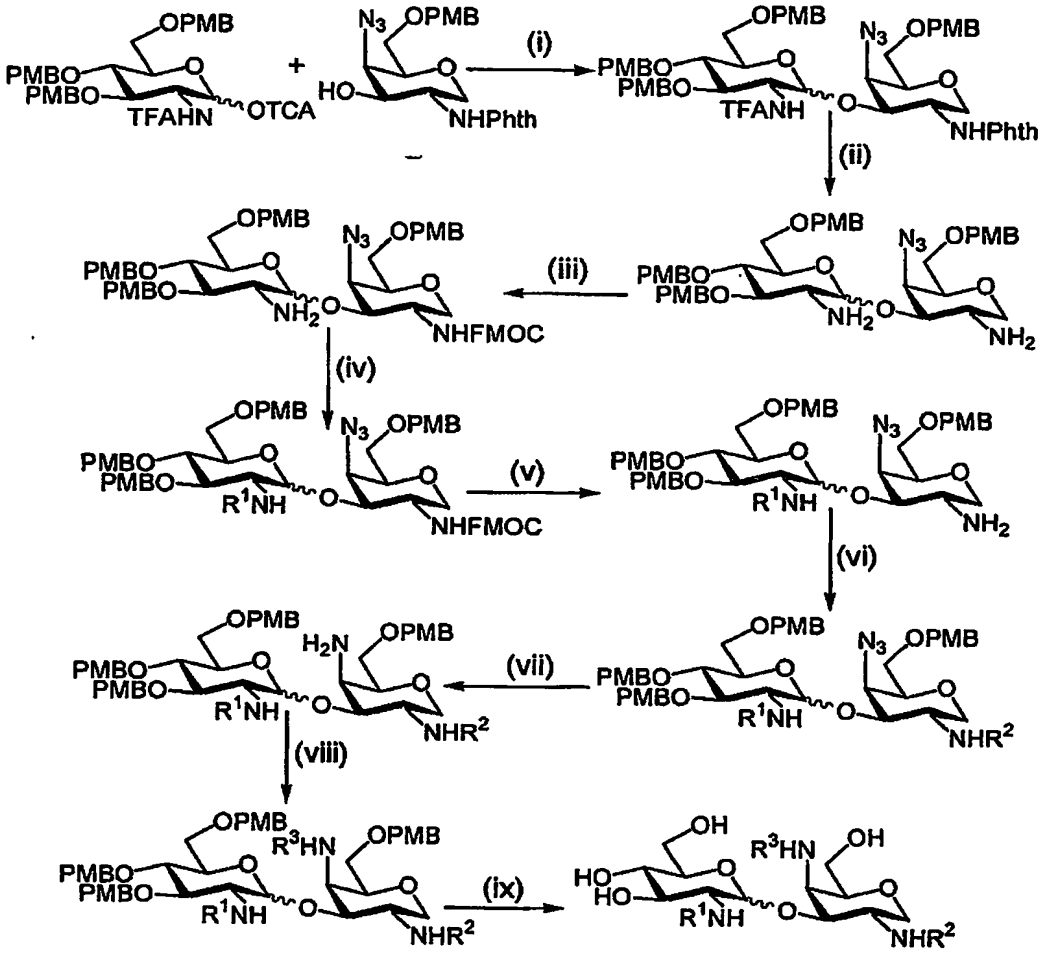
Conditions: (i) Bu_3SnH, toluene, reflux, 2.5hrs, quant.; (ii) NaOMe/MeOH, 2hrs, RT, quant.; (iii) *p*-methoxybenzaldehyde dimethylacetal, *p*-toluenesulphonic acid, DMF, 6hrs, 70°C, 86%; (iv) *p*-chlorobenzoylchloride, pyridine, 1hr, RT, 83%; (v) $NaCNBH_3$, TFA, Mol. sieves 3Å, DMF, 16hrs, 55°C, 93%; (vi) (a) Tf_2O, pyridine, DCM, -20°C, (b) NaN_3, DMF, 1hr, RT, 93%; (vii) NaOMe/MeOH, RT, quant.; (viii) hydrazine, butanol; (ix) (a) Phthalic anhydride, methanol, (b) Ac_2O, pyridine; (x) NaOMe/MeOH.

Example 2: Synthesis of 2-deoxy-2-*N*-Glucopyranoside Donors



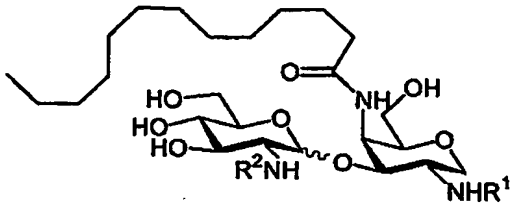
Conditions: (i) NaH, 4-MeOBnCl; (ii) (a) DTT, TEA (b) BzCl, DIPEA; (iii) (a) NBS, (b) Cl_3CN, DBU; (iv) (a) NBS, (b) DTT, TEA; (v) (a) Trifluoroaceticanhydride, (b) $NaHCO_3$; (vi) Cl_3CN, DBU.

Example 3: Formation of a Disaccharide

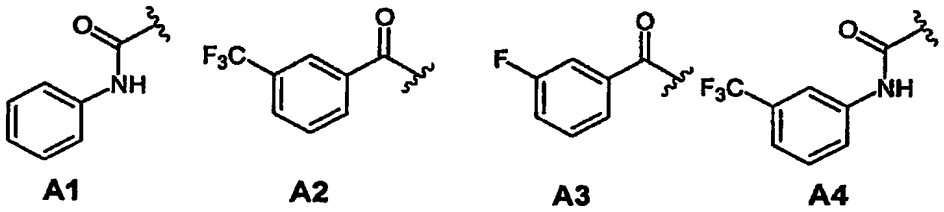


Conditions: (i) TMSOTf, DCE; (ii) hydrazine, heat; (iii) FMOC-Cl, DCE, DIPEA; (iv) (a) ArNCO, or (b) ArC(O)-Cl; (v) piperidine, chloroform; (vi) (a) ArNCO, or (b) ArC(O)-Cl; (vii) DMF, Methanol, ammonium chloride, zinc; (viii) R-CO_2H, HBTU, DIPEA, DMF.

Table 1: Library of Compounds Synthesized



Comp. No	R^1	R^2
1	A1	A1
2	A1	A2
3	A1	A3
4	A4	A4
5	A5	A6
6	A5	A5
7	A7	A7
8	A4	A2
9	A5	A2
10	A5	A3
11	A7	A6
12	A4	A3
13	A7	A2



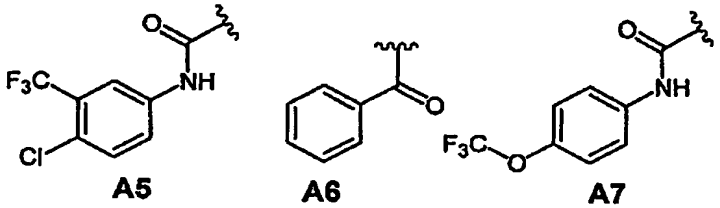


Table 2: Assay Results After Incubation for 20 Hours.

Compound	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5
Tetracyline	complete inhibition	complete inhibition	complete inhibition	complete inhibition	no inhibition
Tetracyline	complete inhibition	complete inhibition	complete inhibition	complete inhibition	no inhibition
Amphotericin B	no inhibition	no inhibition	no inhibition	no inhibition	complete inhibition
Amphotericin B	no inhibition	no inhibition	no inhibition	no inhibition	complete inhibition
1	-	-	-	-	-
2	-	+	-	-	-
3	-	++	-	-	-
4	-	++	-	-	+
5	-	+	-	-	-
6	-	++	-	-	-
7	-	+	-	-	-
8	-	+	-	-	-
9	-	-	-	-	-
10	-	-	-	-	-
11	-	-	-	-	-
12	-	-	-	-	-
13	-	-	-	-	-

KEY

Assay 1: *Escherichia coli (Gram Negative)*
Assay 2: *Staphylococcus aureus (Gram Positive)*
Assay 3: *Klebsiella pneumoniae (Gram Negative)*
Assay 4: *Pseudomonas aeruginosa (Gram Negative)*
Assay 5: *Candida albicans Eukaryotic Yeast*

All compound test concentrations were at 250 μM

"++" indicates an inhibition of greater than 90%

"+" indicates inhibition at greater than 70%

"-" indicates less than 70% inhibition

Table 3: Assay Results After Incubation for 44 Hours.

Compound	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5
Tetracyline	complete inhibition	complete inhibition	complete inhibition	complete inhibition	no inhibition
Tetracyline	complete inhibition	complete inhibition	complete inhibition	complete inhibition	no inhibition
Amphotericin B	no inhibition	no inhibition	no inhibition	no inhibition	complete inhibition
Amphotericin B	no inhibition	no inhibition	no inhibition	no inhibition	complete inhibition
1	-	-	-	-	-
2	-	-	-	-	-
3	-	++	-	-	-
4	-	++	-	-	-
5	-	-	-	-	-
6	-	++	-	-	-
7	-	+	-	-	-
8	-	-	-	-	-
9	-	-	-	-	-
10	-	-	-	-	-
11	-	-	-	-	-
12	-	-	-	-	-
13	-	-	-	-	-

KEY as for Table 2.

Table 4: Selected *In Vitro* Toxicity Data

Compound	Line 1	Line 2	Line 3
3	**	**	*
4	*	**	**
7	**	**	**
13	**	*	*

Line 1: 3T3, mouse embryonic cell line

Line 2: Jurkat, human leukaemia T-cells

Line 3: MCF-7, human breast adenocarcinoma

"** **" indicates no growth inhibition; "**" indicates growth inhibition of less than 20%.

Compounds were tested at a concentration of 25μM

It should be appreciated that various other changes and modifications can be made to the embodiments without departing from the spirit and scope of the invention.

Dated this 17th day of October 2002

Alchemia Pty Ltd

By their Patent Attorneys

CULLEN & CO.

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